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CELL BIOLOGY

Warburg Effect and Redox Balance

A glycolytic enzyme maintains cellular redox homeostasis during metabolic stress.

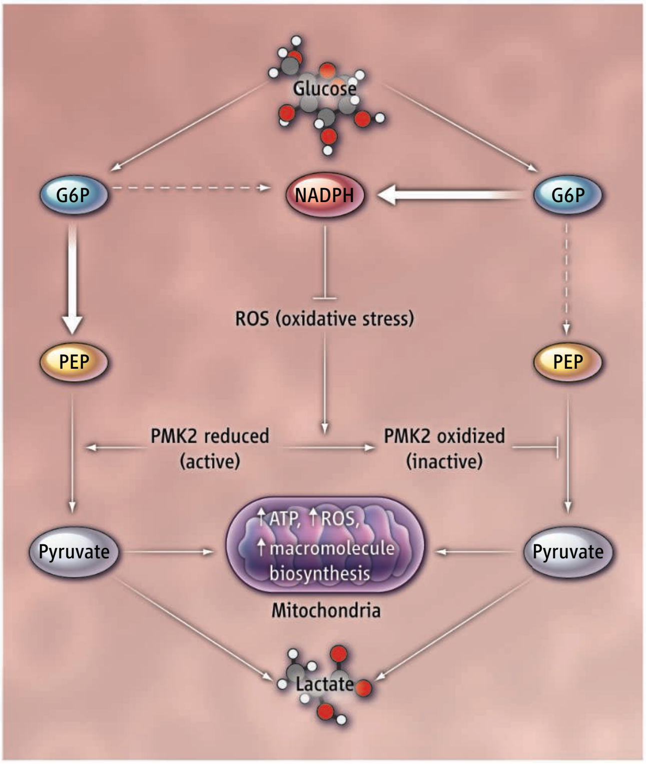
Robert B. Hamanaka[1] and Navdeep S. Chandel[1,2,3]

In the 1920s, German biochemist Otto Warburg demonstrated that tumor cells produce copious amounts of lactate despite the presence of ample oxygen—a phenomenon called aerobic glycolysis or the Warburg effect. Warburg hypothesized that cancer is caused by mitochondrial damage, followed by an increase in glycolysis, which promotes tumorigenesis (*1*). However, mitochondrial defects are rare in cancer, and multiple mechanisms promote glycolysis in tumor cells including growth factor signaling (*2*). On page 1278 in this issue, Anastasiou *et al*. (*3*) show that the enzyme pyruvate kinase M2 (PKM2), an essential regulator of aerobic glycolysis in cancer cells (*4*), has a previously unappreciated role in maintaining cellular redox homeostasis.

Pyruvate kinases catalyze the rate-limiting and adenosine 5′-triphosphate (ATP)–producing step of glycolysis in which phosphoenolpyruvate (PEP) is converted to pyruvate (see the figure). Cancer cells express the M2 isoform of the enzyme rather than its M1 splice variant. Cancer cells engineered to express PKM1 instead of PKM2 display reduced tumor-forming ability, underscoring the importance of PKM2 for cancer progression (*4*). It had been speculated that aerobic glycolysis enables highly proliferating cells to generate ATP from glucose with lower efficiency but at a faster rate than oxidative phosphorylation, thereby meeting metabolic demands associated with proliferation. Paradoxically, however, PKM2 has an enzymatic activity half that of PKM1 and is typically found inactive in vivo. This is due in part to tyrosine phosphorylation that is specific to the M2 isoform, a modification that inhibits its activity (*5*). Thus, cell growth signaling pathways that involve tyrosine kinases promote cellular glucose uptake, yet also diminish glycolytic ATP production by decreasing PKM2 activity.

One model to explain why PKM2 is advantageous to cancer cells takes into account that the slower rate of glycolysis catalyzed by this isoform allows greater diversion of glycolytic intermediates into subsidiary pathways such as the hexosamine, pentose phosphate, and amino acid biosynthetic pathways, thus supporting cellular biomass increase (*2*). Anastasiou *et al.* demonstrate that PKM2 responds not only to cellular growth signals, but to oxidative stress as well. PKM2 is specifically oxidized by hydrogen peroxide (H_2O_2) on cysteine 358 in cancer cells. This diminishes PKM2 activity, decreasing pyruvate formation and increasing flux of glycolytic metabolites into the pentose phosphate pathway. This pathway produces reduced nicotinamide adenine dinucleotide phosphate (NADPH), a crucial source of reducing equivalents for fatty acid synthesis and for the glutathione, peroxiredoxin, and thioredoxin systems that detoxify reactive oxygen species (ROS). Anastasiou *et al*. show that pharmacological activation of PKM2, or expression of a nonoxidizable mutant, led to lower concentrations of cellular reduced glutathione and decreased the ability of cancer cells to detoxify exogenous H_2O_2. The substitution of PKM2 with PMK1 (which is not oxidized by H_2O_2) had a similar effect, indicating that PKM2 participates in a negative feedback loop controlled by cellular oxidative stress. Oxidation of cysteine 358 inactivates PKM2, thereby inducing NADPH production by the pentose phosphate pathway, and increasing cellular redox buffering capacity. This was especially important under hypoxia, a characteristic of most solid tumor microenvironments. Hypoxia increases mitochondrial generation of ROS, which serve as signaling molecules that activate the transcription of genes involved in cellular hypoxic adaptation (*6*). However, an aberrant increase in cellular ROS content above that required for signaling under hypoxia could damage cells. Anastasiou *et al*. show that cancer cells expressing a nonoxidizable PKM2 mutant cannot proliferate under hypoxic conditions, a defect that is rescued by treatment with reduced glutathione.

Cancer cells exhibit high basal levels of oxidative stress due to activation of oncogenes, loss of tumor suppressors, and the effects of the tumor microenvironment (*7*). The increased oxidant concentrations associated with cellular transformation promote tumorigenicity through signaling, but can also damage DNA, proteins, and lipids. Indeed, promoting oxidative stress in cancer cells selectively kills several cancer cell lines (*8*, *9*). Anastasiou *et al*. demonstrate that cancer cells expressing a nonoxidizable PKM2



Buffering ROS. PKM2 participates in a negative feedback loop to control cellular redox homeostasis. Increases in cellular ROS promote the oxidation of PKM2, which inactivates its catalytic activity and promotes diversion of glucose 6-phosphate (G6P) into the pentose phosphate pathway. This pathway produces NADPH, which provides reducing equivalents to cellular antioxidants systems, thereby increasing cellular redox buffering capacity.

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mutant form smaller tumors in mice than cells expressing wild-type PKM2. This difference in growth was rescued by treatment of mice with the antioxidant *N*-acetylcysteine, which is a precursor for glutathione synthesis. The results suggest that small-molecule activators of PKM2 that limit the ability of glycolytic intermediates to fuel the pentose phosphate pathway, coupled with radiation or chemotherapeutic drugs that increase oxidative stress, may promote high levels of oxidative stress which are toxic to cancer cells.

PKM2 is also expressed in nontransformed cells, including stem cells (*10*), which are exquisitely sensitive to oxidative stress. Stem cells proliferate slowly, reside in hypoxic niches, and display robust glucose metabolism (*11*). This high rate of glucose metabolism is supported by the transcription factor HIF-1, which promotes glycolysis while inhibiting oxidative phosphorylation. Interestingly, PKM2 localizes to the nucleus, where it interacts with HIF-1 and promotes the expression of HIF-1 target genes (*12*). PKM2 also interacts with -catenin and OCT-4, two factors important for stem cell maintenance, highlighting the diverse roles of PKM2 in this capacity (*13*, *14*). It is unclear whether these nuclear functions of PKM2 are affected by cysteine oxidation, or whether the redox buffering role of PKM2 is important in other nontransformed cells that express PKM2.

Regulation of the pentose phosphate pathway to promote cellular redox balance is not limited to mammals. The yeast *Saccharomyces cerevisiae* switches pyruvate kinase isoforms from the high-flux PYK1 when grown on fermentable carbon sources to the low-flux PYK2 when grown on oxidizable carbon sources. Low pyruvate kinase activity in yeast promotes flux through the pentose phosphate pathway, promoting NADPH production and protecting yeast from oxidative damage caused by mitochondrial ROS generation during respiration (*15*). Thus, expression of low-activity isoforms of pyruvate kinase seems to be an evolutionarily conserved mechanism to promote cellular redox homeostasis.

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SOCIOLOGY

Experimenting with Buddies

Internet experiments start to unravel the role of social networks in the spread of behavior in society.

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Go to any social gathering in your neighborhood and you will notice that people interact mostly with others who are similar in terms of age, gender, race, attributes, and behaviors. This tendency of people to have similar friends—known as homophily—is one of the most pervasive features of social networks (*1*). A key question is how much of the homophily in behavior can be attributed to social diffusion, that is, direct causal influence of one person on another through social ties (*2*, *3*). Results from two clever Internet experiments reported by Centola last year (*4*) and on page 1269 of this issue (*5*) shed light on how the particular arrangement of social ties promotes social diffusion.

Observational studies on real-world behavioral diffusion cannot disentangle social contagion, homophily, and friendship formation (*6*, *7*). Therefore, social scientists would like to conduct randomized controlled experiments instead, as is standard in biomedical research. Although some network experiments have been carried out (*8*–*10*), Centola's Internet experiments come closest to the ideal of a non-artificial randomized experiment, in which the network structure is fully controlled by the experimenter.

For his studies, Centola devised a professional-looking social network Web site to promote health and fitness. Subjects were attracted to this site through advertisements on health Web sites. At registration, each subject chose a username and avatar and was assigned six other subjects, his or her "health buddies," whose characteristics and activities the subject could observe during participation. When a subject started a new activity, his or her buddies were invited to also participate in this activity. The experimenter controlled the matching of health buddies and ensured that, after introduction, subjects only learned about the new activity through their health buddies. This setup enforced social diffusion of the new activity and allowed the experimenter to analyze the effect of different health buddy assignments on the level of social diffusion.

The experimental design in last year's study randomly assigned subjects to two conditions: one in which the matching of health buddies formed a clustered network (see the figure, panel A), and another in which the matching formed a random network (see the figure, panel B). In each network, health buddies of an initial dummy subject received a personalized invitation to register for a health forum. Acceptance triggered a new round of invitations to the health buddies of those who registered, and so on, leading to diffusion of the health forum registration through the network.

The results were surprising. Centola found that diffusion reached more subjects in the clustered networks than in the random networks, whereas standard percolation or epidemiological diffusion models would predict the opposite. However, in these models, one node can infect each of its neighbors with equal probability, whereas in social diffusion, two or more "infected" friends are often required to persuade an exposed subject to adopt his or her friends' behavior.

Centola's present results are equally surprising. This time, buddy matching imposed exactly the same network structure on both conditions, but levels of homophily differed: In one condition, no homophily bias was

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ERRATUM

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Perspectives: "Warburg effect and redox balance" by R. B. Hamanaka and N. S. Chandel (2 December 2011, p. 1219). In the figure, PMK2 should be PKM2.

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